

SEQUENCE LISTING

<110> Agriculture Agroalimentaire Canada
Côté, Jean-Charles
Mizuki, Eiichi
Akao, Tetsuyuki
Jung, Yong-Chul

<120> A NOVEL BACILLUS THURINGIENSIS STRAIN, CRYSTAL GENE AND CRYSTAL
PROTEIN AND USES THEREOF

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<140> 2,410,153

<141> 2002-12-05

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<170> PatentIn version 3.2

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Gln Ser Asp Leu Gln Tyr Pro Val Leu Thr Leu Pro Leu Arg Ala Gln
 35 40 45

Ala Cys Val Met His Leu Met Leu Leu Lys Asp Ala Thr Thr Ser Val
 50 55 60

Trp Gly Gln Gln Ile Asp Ser Gln Gln Leu Asn Gly Tyr Lys Ala Glu
 65 70 75 80

Leu Ile Arg Leu Ile Lys Val Tyr Thr Asn Asp Val Asn Thr Thr Tyr
 85 90 95

Asn Gln Gly Leu Glu Leu Glu Lys Ala Lys Pro Leu Asn Tyr Ser Asp
 100 105 110

Pro Glu Glu Tyr Leu Gln Ala Gly Arg Pro Asp Ile Ser Val Leu Arg
 115 120 125

Ser Asn Phe Lys Glu Val Met Lys Trp Asn Xaa Val Ala Lys Tyr Lys
 130 135 140

Arg Gly Met Ala Met Ser Ala Leu Ser Leu Ala Ala Leu Phe Pro Thr
 145 150 155 160

Phe Gly Pro Asn Tyr Pro Lys Gln Ala Leu Lys Val Val Gln Ser Arg
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Gln Ile Phe Ala Pro Val Ile Gly Ile Pro Gly Gly Ile Thr Ser Gln
 180 185 190

Asp Xaa Xaa Xaa Thr Phe Gly Ser Met Arg Phe Asp Val Lys Thr Tyr
 195 200 205

Asp Gln Ile Asp Ala Leu Arg Xaa Leu Met Glu Leu Tyr Ile Gln Pro
 210 215 220

Leu Lys Ser Ala Tyr Phe Xaa Ile Tyr Glu Ser Asp Trp Lys Val Arg
 225 230 235 240

Ala Thr Tyr Val Asn Asp Tyr Ile Gly Lys Arg Gly Ser Asn Thr Gly
 245 250 255

Xaa Ala Trp Xaa Met Trp Ser Ser Asp Pro Ser Xaa Ile Tyr Thr Ser
 260 265 270

Ala Leu Gly Ala Ala Gly Tyr Ala Pro Asn Val Val Gly Val Arg Tyr
 275 280 285

Ser His Gly Gly Ser Tyr Thr Lys Gly Met Ala Pro Xaa Asn Thr Asn
 290 295 300

Ala Tyr Ala Pro Phe Glu Phe Lys Tyr Pro Gly Tyr Lys Leu His Ser
 305 310 315 320

Val Ser Ala Tyr Gly Leu Ser Lys Ala Pro Asp Xaa Ala Asp Ser Val
 325 330 335

Met Phe Gly Phe Arg Pro Val Leu Leu Glu Asn Glu Ala Asn Gln Leu
 340 345 350

Leu Thr Asp Thr Ala Leu Gln Ile Pro Ala Glu Ile Gly Ile Thr Asp
 355 360 365

Val Val Pro Ala Phe Gly Arg Thr Glu Glu Pro Ile Asn Gly Gln Asp
 370 375 380

Ala Ile Xaa Ile Trp Glu Ser Phe Thr Ser Gly Phe Gly Phe Thr Tyr
 385 390 395 400

Thr Val Asp Ser Pro Gln Lys Gln Lys Tyr Lys Ile Ile Tyr Arg Ile
 405 410 415

Ala Asn Asn Leu Ser Ala Ser Thr Val Ser Leu Thr Tyr Asn Asn Gln
 420 425 430

Thr Phe Phe Thr Asp Ile Leu Asn Thr Ser Leu Asp Pro Asn Gly Val
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Arg Gly Asn Tyr Gly Ser Tyr Thr Leu Val Glu Gly Pro Ile Ile Glu
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Phe Ala Ile Asp Ser Ile Ile Phe Ser Pro Val Xaa
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Lys Phe Xaa Asn Gln Glu Phe Xaa Asp Asn Xaa Tyr Gln His Ser Asp
 50 55 60

Val Ser Asn Ser Tyr Gln Asn Met Lys Thr Glu Ile Val Asn Thr Asp
 65 70 75 80

Leu Pro Tyr Asn Thr Asn Xaa Ile Asn Ser Met Arg Asn Thr Leu Cys
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Xaa Asp Leu Pro Pro Glu Thr Asn Met Ser Ile Tyr Asp Asn Leu Arg
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Ser Thr Val Thr Val Pro Ser Phe Ser Asn Gln Phe Asp Pro Ile Lys
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Phe Leu His Asp Ile Glu Ile Ala Ile Xaa Thr Gly Ser Phe Ser Ala
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Leu Thr Gln Ser Asn Met Asn Gln Gly Gly Thr Asp Ile Xaa Pro Met
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Leu Ile Ser Thr Phe Phe Lys Val Ala Xaa Ser Leu Leu Pro Phe Pro
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Leu Ser Ser Leu Gly Ala Leu Ala Ser Phe Tyr Val Thr Asp Ser Gln
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Thr Gly Ala Met Ala Asn Leu Trp Arg Gln Met Val Asp Tyr Val Glu
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Lys Arg Ile Asp Ser Lys Ile Leu Asp Tyr His Asn Phe Ile Met Gly
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 225 230 235 240

Val Lys Ile Phe Glu Asn Asp Met Asn Arg Xaa Ala Glu Pro Pro Ser
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Thr Gly Val Ile Thr Gln Phe Arg Ile Leu Asn Asp Asn Phe Ile Lys
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Tyr Ile Ala Lys Leu Gln Phe Ser Thr Asn Gln Ser Asp Leu Gln Tyr
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Ser Gln Gln Leu Asn Gly Tyr Lys Ala Glu Leu Ile Arg Leu Ile Lys
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Val Tyr Thr Asn Asp Val Asn Thr Thr Tyr Asn Gln Gly Leu Glu Leu
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Lys Gln Ala Leu Lys Val Val Gln Ser Arg Gln Ile Phe Ala Pro Val
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Ile Gly Ile Pro Gly Gly Ile Thr Ser Gln Asp Xaa Xaa Xaa Thr Phe
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Gly Ser Met Arg Phe Asp Val Lys Thr Tyr Asp Gln Ile Asp Ala Leu
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Xaa Ile Tyr Glu Ser Asp Trp Lys Val Arg Ala Thr Tyr Val Asn Asp
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Tyr Ile Gly Lys Arg Gly Ser Asn Thr Gly Xaa Ala Trp Xaa Met Trp
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Ser Ser Asp Pro Ser Xaa Ile Tyr Thr Ser Ala Leu Gly Ala Ala Gly
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Tyr Ala Pro Asn Val Val Gly Val Arg Tyr Ser His Gly Gly Ser Tyr
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Thr Lys Gly Met Ala Pro Xaa Asn Thr Asn Ala Tyr Ala Pro Phe Glu
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Phe Lys Tyr Pro Gly Tyr Lys Leu His Ser Val Ser Ala Tyr Gly Leu
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Ser Lys Ala Pro Asp Xaa Ala Asp Ser Val Met Phe Gly Phe Arg Pro
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Val Leu Leu Glu Asn Glu Ala Asn Gln Leu Leu Thr Asp Thr Ala Leu
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Gln Ile Pro Ala Glu Ile Gly Ile Thr Asp Val Val Pro Ala Phe Gly
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Arg Thr Glu Glu Pro Ile Asn Gly Gln Asp Ala Ile Xaa Ile Trp Glu
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Lys Gln Lys Tyr Lys Ile Ile Tyr Arg Ile Ala Asn Asn Leu Ser Ala
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Ser Thr Val Ser Leu Thr Tyr Asn Asn Gln Thr Phe Phe Thr Asp Ile
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Tyr Thr Leu Val Glu Gly Pro Ile Ile Glu Phe Ser Gln Gly Thr Asn
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Gln Ser Asp Leu Gln Tyr Pro Val Leu Thr Leu Pro Leu Arg Ala Gln
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Ala Cys Val Met His Leu Met Leu Leu Lys Asp Ala Thr Thr Ser Val
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Trp Gly Gln Gln Ile Asp Ser Gln Gln Leu Asn Gly Tyr Lys Ala Glu
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Leu Ile Arg Leu Ile Lys Val Tyr Thr Asn Asp Val Asn Thr Thr Tyr
 85 90 95

Asn Gln Gly Leu Glu Leu Glu Lys Ala Lys Pro Leu Asn Tyr Ser Asp
 100 105 110

Pro Glu Glu Tyr Leu Gln Ala Gly Arg Pro Asp Ile Ser Val Leu Arg
 115 120 125

Ser Asn Phe Lys Glu Val Met Lys Trp Asn Xaa Val Ala Lys Tyr Lys
 130 135 140

Arg Gly Met Ala Met Ser Ala Leu Ser Leu Ala Ala Leu Phe Pro Thr
 145 150 155 160

Phe Gly Pro Asn Tyr Pro Lys Gln Ala Leu Lys Val Val Gln Ser Arg
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Gln Ile Phe Ala Pro Val Ile Gly Ile Pro Gly Gly Ile Thr Ser Gln
 180 185 190

Asp Xaa Xaa Xaa Thr Phe Gly Ser Met Arg Phe Asp Val Lys Thr Tyr
 195 200 205

Asp Gln Ile Asp Ala Leu Arg Xaa Leu Met Glu Leu Tyr Ile Gln Pro
 210 215 220

Leu Lys Ser Ala Tyr Phe Xaa Ile Tyr Glu Ser Asp Trp Lys Val Arg
 225 230 235 240

Ala Thr Tyr Val Asn Asp Tyr Ile Gly Lys Arg Gly Ser Asn Thr Gly
 245 250 255

Xaa Ala Trp Xaa Met Trp Ser Ser Asp Pro Ser Xaa Ile Tyr Thr Ser
 260 265 270

Ala Leu Gly Ala Ala Gly Tyr Ala Pro Asn Val Val Gly Val Arg Tyr
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Ser His Gly Gly Ser Tyr Thr Lys Gly Met Ala Pro Xaa Asn Thr Asn
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Ala Tyr Ala Pro Phe Glu Phe Lys Tyr Pro Gly Tyr Lys Leu His Ser
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Val Ser Ala Tyr Gly Leu Ser Lys Ala Pro Asp Xaa Ala Asp Ser Val
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Met Phe Gly Phe Arg Pro Val Leu Leu Glu Asn Glu Ala Asn Gln Leu
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Leu Thr Asp Thr Ala Leu Gln Ile Pro Ala Glu Ile Gly Ile Thr Asp
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Val Val Pro Ala Phe Gly Arg Thr Glu Glu Pro Ile Asn Gly Gln Asp
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Ala Ile Xaa Ile Trp Glu Ser Phe Thr Ser Gly Phe Gly Phe Thr Tyr
 385 390 395 400

Thr Val Asp Ser Pro Gln Lys Gln Lys Tyr Lys Ile Ile Tyr Arg Ile
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Ala Asn Asn Leu Ser Ala Ser Thr Val Ser Leu Thr Tyr Asn Asn Gln
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Thr Phe Phe Thr Asp Ile Leu Asn Thr Ser Leu Asp Pro Asn Gly Val
 435 440 445

Arg Gly Asn Tyr Gly Ser Tyr Thr Leu Val Glu Gly Pro Ile Ile Glu
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 <211> 723
 <212> PRT
 <213> Bacillus thuringiensis

<400> 18

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Asn Asn Gln Glu Leu Ile Thr Lys Ser Ser Ser Phe Tyr Ser Asp Thr
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Thr Asn Glu Asn Ala Lys Asn Tyr His Pro Ile Glu Gln Asp Ile Leu
 35 40 45

Lys Phe Thr Asn Gln Glu Phe Ser Asp Asn His Tyr Gln His Ser Asp
 50 55 60

Val Ser Asn Asp Ile Asn Ser Met Arg Asn Thr Leu Cys Lys Asp Leu
 65 70 75 80

Pro Pro Glu Thr Asn Met Ser Ile Tyr Asp Asn Leu Arg Ser Thr Val
 85 90 95

Thr Val Pro Ser Phe Ser Asn Gln Phe Asp Pro Ile Lys Phe Leu His
 100 105 110

Asp Ile Glu Ile Ala Ile Gln Thr Gly Ser Phe Ser Ala Leu Thr Gln
 115 120 125

Ser Asn Met Asn Gln Gly Gly Thr Asp Ile Asn Pro Met Leu Ile Ser
 130 135 140

Thr Phe Phe Lys Val Ala Ser Ser Leu Leu Pro Phe Pro Leu Ser Ser
 145 150 155 160

Leu Gly Ala Leu Ala Ser Phe Tyr Val Thr Asp Ser Gln Thr Gly Ala
 165 170 175

Met Ala Asn Leu Trp Arg Gln Met Val Asp Tyr Val Glu Lys Arg Ile
 180 185 190

Asp Ser Lys Ile Leu Asp Tyr His Asn Phe Ile Met Gly Ala Glu Leu
 195 200 205

Ala Ala Leu Asn Ala Ser Leu Lys Glu Tyr Ala Arg Val Val Lys Ile
 210 215 220

Phe Glu Asn Asp Met Asn Arg Met Ala Glu Pro Pro Ser Thr Gly Val
225 230 235 240

Ile Thr Gln Phe Arg Ile Leu Asn Asp Asn Phe Ile Lys Tyr Ile Ala
245 250 255

Lys Leu Gln Phe Ser Thr Asn Gln Ser Asp Leu Gln Tyr Pro Val Leu
260 265 270

Thr Leu Pro Leu Arg Ala Gln Ala Cys Val Met His Leu Met Leu Leu
275 280 285

Lys Asp Ala Thr Thr Ser Val Trp Gly Gln Gln Ile Asp Ser Gln Gln
290 295 300

Leu Asn Gly Tyr Lys Ala Glu Leu Ile Arg Leu Ile Lys Val Tyr Thr
305 310 315 320

Asn Asp Val Asn Thr Thr Tyr Asn Gln Gly Leu Glu Leu Glu Lys Ala
325 330 335

Lys Pro Leu Asn Tyr Ser Asp Pro Glu Glu Tyr Leu Gln Ala Gly Arg
340 345 350

Pro Asp Ile Ser Val Leu Arg Ser Asn Phe Lys Glu Val Met Lys Trp
355 360 365

Asn Arg Val Ala Lys Tyr Lys Arg Gly Met Ala Met Ser Ala Leu Ser
370 375 380

Leu Ala Ala Leu Phe Pro Thr Phe Gly Pro Asn Tyr Pro Lys Gln Ala
385 390 395 400

Leu Lys Val Val Gln Ser Arg Gln Ile Phe Ala Pro Val Ile Gly Ile
405 410 415

Pro Gly Gly Ile Thr Ser Gln Asp His Ser Gly Thr Phe Gly Ser Met
420 425 430

Arg Phe Asp Val Lys Thr Tyr Asp Gln Ile Asp Ala Leu Arg Arg Leu
435 440 445

Met Glu Leu Tyr Ile Gln Pro Leu Lys Ser Ala Tyr Phe Tyr Ile Tyr
450 455 460

Glu Ser Asp Trp Lys Val Arg Ala Thr Tyr Val Asn Asp Tyr Ile Gly
 465 470 475 480

Lys Arg Gly Ser Asn Thr Gly Leu Ala Trp Gly Met Trp Ser Ser Asp
 485 490 495

Pro Ser Val Ile Tyr Thr Ser Ala Leu Gly Ala Ala Gly Tyr Ala Pro
 500 505 510

Asn Val Val Gly Val Arg Tyr Ser His Gly Gly Ser Tyr Thr Lys Gly
 515 520 525

Met Ala Pro Pro Asn Thr Asn Ala Tyr Ala Pro Phe Glu Phe Lys Tyr
 530 535 540

Pro Gly Tyr Lys Leu His Ser Val Ser Ala Tyr Gly Leu Ser Lys Ala
 545 550 555 560

Pro Asp Thr Ala Asp Ser Val Met Phe Gly Phe Arg Pro Val Leu Leu
 565 570 575

Glu Asn Glu Ala Asn Gln Leu Leu Thr Asp Thr Ala Leu Gln Ile Pro
 580 585 590

Ala Glu Ile Gly Ile Thr Asp Val Val Pro Ala Phe Gly Arg Thr Glu
 595 600 605

Glu Pro Ile Asn Gly Gln Asp Ala Ile Ile Ile Trp Glu Ser Phe Thr
 610 615 620

Ser Gly Phe Gly Phe Thr Tyr Thr Val Asp Ser Pro Gln Lys Gln Lys
 625 630 635 640

Tyr Lys Ile Ile Tyr Arg Ile Ala Asn Asn Leu Ser Ala Ser Thr Val
 645 650 655

Ser Leu Thr Tyr Asn Asn Gln Thr Phe Phe Thr Asp Ile Leu Asn Thr
 660 665 670

Ser Leu Asp Pro Asn Gly Val Arg Gly Asn Tyr Gly Ser Tyr Thr Leu
 675 680 685

Val Glu Gly Pro Ile Ile Glu Phe Ser Gln Gly Thr Asn Ile Phe Lys
 690 695 700

Leu Arg Ser Gln Lys Gly Glu Phe Ala Ile Asp Ser Ile Ile Phe Ser
705 710 715 720

Pro Val Ser

any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), (h), (i) (j), (k), (l), (m) and (n); and

- (p) a nucleotide sequence which hybridizes under high stringency conditions to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), (h), (i) (j), (k), (l), (m), (n) and (o).

3. An isolated polypeptide comprising a sequence selected from the group consisting of:

- (a) an amino acid as set forth in SEQ ID NO: 2;
- (b) an amino acid sequence in SEQ ID NO: 8;
- (c) an amino acid sequence of a crystal protein contained in the *bacillus thuringiensis* strain in the deposit at the International Depository Authority of Health Canada in Winnipeg under accession number IDAC010201-5;
- (d) a crystal protein comprising the amino acid sequence in SEQ ID NO: 10;
- (e) a crystal protein having at least 94% identity with the complete amino acid sequence in SEQ ID NO: 2, with the proviso that said crystal protein is not constituted of SEQ ID NO: 18 ;
- (f) a crystal protein having at least 97% identity with the complete amino acid sequence in SEQ ID NO: 8, with the proviso that said crystal protein is not constituted of the amino acid sequence at positions 232 to 723 of SEQ ID NO: 18;
- (g) a crystal protein cytotoxic against at least one human cancer cell and encoded by a nucleotide sequence having at least 98% identity with the complete sequence in SEQ ID NO: 9, with the proviso that said nucleotide sequence does encode the amino acid sequence at positions 232 to 723 of SEQ ID NO: 18.